

Neural Control of Flight Muscle Differentiation in the Fly, *Sarcophaga bullata*

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ABSTRACT—Myoblasts derived from imaginal discs together with the degenerating larval muscles contribute to the formation of flight muscles in adult flies. Severance of the mesothoracic larval nerve in the freshly formed prepupa results in the absence of entire flight musculature on the operated side of the adult fly. Histological observations reveal very early stages of muscle differentiation in the form of association of myoblasts with degenerating larval muscle around day 3 of pupal development. Further differentiation is inhibited by nerve transection leading to the eventual degeneration of all muscles on the operated side. These results indicate that insect flight muscles are dependent on nerves from the early stages of their differentiation.

INTRODUCTION

Although the importance of innervation in the differentiation of vertebrate muscle has been established by several investigations [1–6], insects that undergo complete metamorphosis are better suited for such studies because of precise timing in the differentiation of adult muscles. When a crawling maggot such as that of a fly, metamorphoses into a flying insect there is a considerable reorganization of its locomotor apparatus. The larval muscles are histolysed and replaced by imaginal muscles. The nervous system too is remodelled accordingly. Such a metamorphosing system is very convenient for the study of neural control of muscle differentiation.

In one of his pioneering studies on the influence of nerves on muscle differentiation Kopeć [7] removed the thoracic ganglia from gypsy moth caterpillars. This resulted in the development of adults without thoracic muscles. Similar results were also obtained for silkmths by Williams and Schneiderman [8]. Nüesch extended these studies to single nerves innervating specific muscles of the

moth, *Antheraea pernyi* and concluded that innervation is essential for the completion of muscle differentiation [9]. We have studied the influence of nerves in the differentiation of muscles in a different order of insects namely, Diptera. As in other flies of Diptera, the adult *Sarcophaga bullata* has six pairs of dorsal longitudinal muscles (DLM), part of the indirect flight musculature, that increase the height of the thoracic box during flight and thus depress the wings. These muscles are innervated by the posterior dorsal mesothoracic nerve (PDMN) from the thoracic ganglion [10]. In this report we have examined the role of PDMN in the differentiation of DLM.

MATERIALS AND METHODS

The fleshfly *Sarcophaga bullata* was reared in the laboratory under constant conditions of temperature (25°C) and photoperiod (16L:8D). The adult flies were fed with sugar and water *ad libitum*. The larvae were raised in fresh beef liver. Post-feeding mature third instar larvae were collected and used for experiments within two hr after pupariation.

Denervation In the mature larva there are groups of embryonic cells enclosed within non-cellular peripodial membranes. These are called

imaginal discs which differentiate into adult structures during metamorphosis. Each disc is connected terminally to the larval epidermis via slender epithelial stalk. Most of the discs also have a basal stalk connecting them to the larval central nervous system. This stalk also contains a larval nerve [11]. Thus the pro- and mesothoracic leg discs are connected to the ventral side of the ganglion by pro- and mesothoracic larval nerves which also send off branches to innervate the larval thoracic muscles [12]. Since it has been known that (a) some larval thoracic muscles contribute to the formation of adult DLM [13], (b) larval neurons are remodelled into adult nerve cells [14], and (c) the PDMN innervating the DLM of the adult originates in the mesothoracic neuromere [15], we hypothesised that the nerve branch of the larval

mesothoracic nerve is transformed into PDMN of the adult and is essential for the differentiation of adult DLM. This larval nerve was transected as follows: a triangular cut was made in the puparium on the anterior ventral side (segments 4–5) of the 1–2 hr old prepupa. While lifting the puparial flap, a fine iridectomy scissors was introduced inside and the basal disc stalk along with its nerve branch of the mesothoracic leg disc was severed (Fig. 1A, top). The epithelial stalk was left intact. The window was closed back with the triangular flap and the drying of the hemolymph sealed the wound. In the sham operated controls, the entire mesothoracic leg disc was extirpated (Fig. 1A, bottom) leaving the larval nerve connection intact as described by Nässel *et al.* [16]. A single oblique cut was made on the anterior ventral (segments 3–

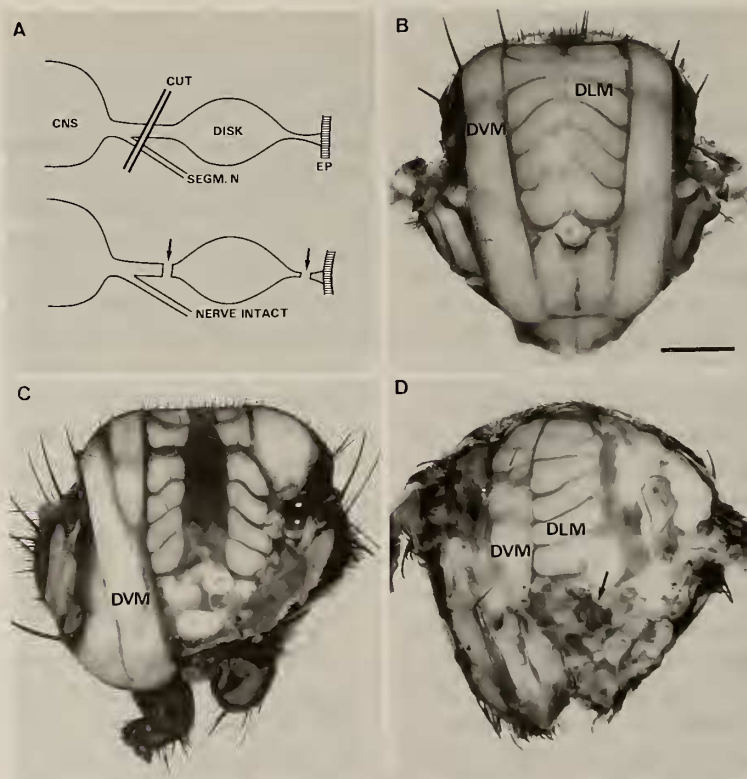


FIG. 1. Nerve transection procedure and its effects on adult flies. Abbreviations: DVM: Dorsoventral flight muscles; DLM: Dorsal longitudinal muscles; EP: Epithelium; Segm. N: Segmental nerve. Scale bar in B, C and D is the same = 1 mm. A. Top: Transection of mesothoracic leg disc stalk along with the larval mesothoracic nerve. Bottom: Extirpation of mesothoracic leg disc (sham-operation). B. Cross section of a thorax from an unoperated control fly. C. Cross section of a thorax from sham operated (mesothoracic leg disc extirpated) fly. D: Cross section of a thorax from nerve transected fly. Note the absence of flight muscles on the right side.

4) side of the freshly formed prepupa. Gentle pressure on one side externalized the mesothoracic leg disc which was then detached from its attachments on both ends. The mesothoracic larval nerve branch that innervates the larval muscles was left intact. Upon completion of metamorphosis (about 12 days at 25°C) the condition of their flight muscles was examined by simply slicing the thoraces as well as after histological staining of 8

μm thick serial paraffin sections.

RESULTS

Control 1 (unoperated flies)

Figure 1B is a cross section of a thorax from an unoperated adult fly. As in other cyclorrhaphous diptera it contains six pairs of DLM which are the main depressors of the wings during flight. These

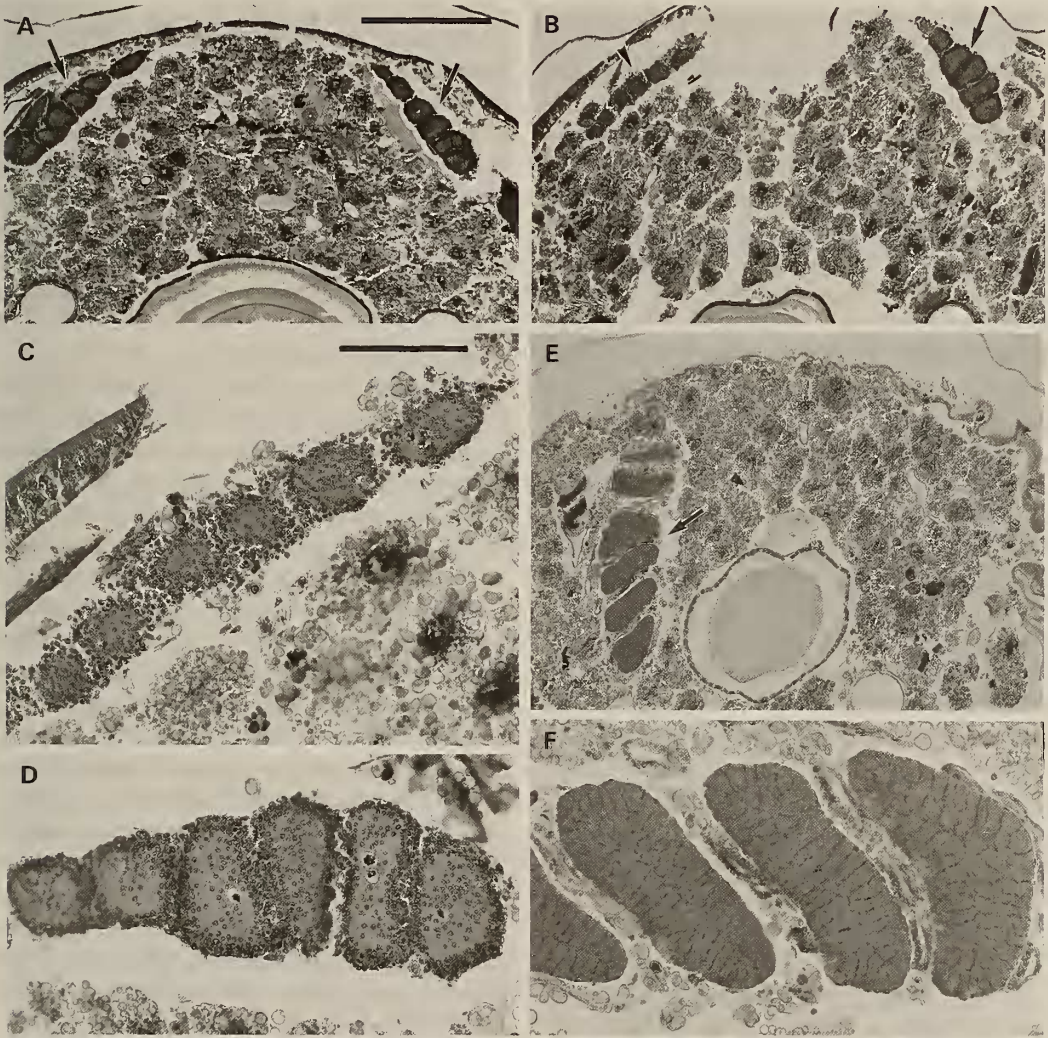


FIG. 2. Chronological stages of muscle degeneration after nerve transection. Magnification in A, B and E is the same. (scale bar = 400 μm). C, D and F are of same magnification (scale bar = 100 μm). A. 3 day old pupa showing DLM on both sides (arrows). B. 3.5 to 4 day old pupa. Note degenerating DLM on left side (arrow head). C. Enlarged view of degenerating DLM from 2B. D. Enlarged view of normal DLM from 2B. E. 7 day old pupa operated on the right side showing the DLM on left side only (arrow). F. Enlarged view of normal DLM from 2E.

muscles extend from the anterior half of alinotum to the post notum and second phragma.

Control II (sham operated flies)

The mesothoracic leg extirpated prepupae metamorphose into five-legged flies. Besides the mesothoracic leg, these flies also lack certain sclerites on the operated side such as the mesosternum. Nevertheless, all six pairs of DLM develop quite normally (Fig. 1C). However, the dorso-ventral muscles (DVM) are absent on the operated side.

Experimental flies

More than 75% of the pupae survive the operation and at least 35 flies that completed metamorphosis were used for examination. Upon transection of the basal stalk along with the attached larval nerve of the mesothoracic leg disc the pupae metamorphosed quite normally with all six legs and associated sclerites. However, they invariably failed to eclose unaided. When the flies were taken out of their puparia one could notice some external color difference between the right and left halves of the dorsal thorax. One half was paler than the other half. This was due to the complete absence of the entire set of fibrillar flight muscles on the operated side which could be seen through the as yet untanned cuticle. The muscleless half of the thoracic box was filled with fat body (Fig. 1D). The thoracic ganglion too was slightly abnormal in such flies and the PDMN was absent on the left side.

At what time during the course of development do muscles need innervation? In the experimental flies do the muscles differentiate first and then degenerate or they fail to differentiate altogether due to lack of innervation? To answer these questions, nerve transected pupae (10 per stage) were histologically examined at different stages of development. Three days after pupariation the larval mesothoracic muscles had cleaved longitudinally into six bundles on either (right and left) side of the thorax with the myoblasts lined up around them. In a cross section one can see these bundles just beneath the dorsal epidermis (Fig. 2A). Within the next 12–24 hr (3–4 day old pupa) these incipient muscle bundles start to degenerate on the operated side (Fig. 2B and 2C) and by the next day they have completely disappeared from the nerve

transected side. Figure 2E is a 7 day old pupa showing the flight muscles on the unoperated side.

DISCUSSION

By selective transection of larval mesothoracic nerve the present study confirms the earlier reports on moths by Kopeć [7] and Williams and Schneiderman [8] and demonstrates the importance of innervation for the differentiation of thoracic flight muscles in the fleshfly, *Sarcophaga bullata*. However, our results are slightly different from those obtained by Nüesch [9] after denervation of developing muscles in diapausing pupae of saturniid moth *Antheraea pernyi* where the nerve transection resulted in retardation of muscle differentiation with fewer nuclei and thinner, shorter muscle fibers. This difference in nerve influence may be due to the timing of denervation. In the moth the operation was performed in the pupal stage, whereas in the fly it was done much earlier in the freshly formed prepupa.

The indirect flight muscles of holometabolous insects develop from myoblasts derived from leg imaginal discs [17, 18] in association with degenerating larval intersegmental muscles [19, 20]. The residual larval intersegmental muscles of the mesothorax form a scaffolding around which the myoblasts line up in the form of compact columns and subsequently differentiate into myofibrils [21]. Upon nerve transection in the prepupa the adult thorax is completely devoid of flight muscles on the denervated side. Our histological preparations indicate that from the moment the myoblasts associate with the longitudinally cleaved larval muscles their further differentiation is dependent on innervation because, on the nerve transected side the muscles seem to start degenerating soon after this stage (Fig. 2B). The precise nature of degeneration is difficult to discern from the current histological observations. Only future studies using ultrastructural and immunocytochemical techniques will shed more light in this regard.

The importance of motor innervation during early stages of muscle differentiation is well documented for vertebrate embryos [1]. In chick embryos motor neuron growth cones associate with muscle forming mesodermal cells even before

myotube formation [22]. Destruction of motor neurons by bungarotoxin inhibits myotube formation in rat skeletal muscles [2]. It was suggested that motor neuron terminals have some trophic or inductive influence on myogenic cells [4, 5]. Accumulation of myosin in developing limb bud musculature of quail embryos is nerve dependent [23]. In grasshopper embryos too the motor neurons contact muscle pioneers very early in development [24] and may start influencing them from the very beginning.

The indirect flight muscles of the adult fleshfly *Sarcophaga bullata* are innervated by PDMN [10]. It is interesting to note that this nerve is missing on the operated side. The absence of muscles could not have caused the degeneration of PDMN because insect motor neurons are able to survive in the absence of their targets [25] and innervate inappropriate muscles [16, 26]. Therefore, it is tempting to suggest that the larval nerve which innervates the mesothoracic muscles of the larva perhaps becomes transformed into PDMN of the adult. Thus, the PDMN has a dual role; first, during metamorphosis it influences the flight muscle differentiation and then, in the adult fly it controls the function of these muscles. Transection of the larval mesothoracic nerve results in the absence of PDMN in the adult. Such a transformation of larval nerve innervating the dorsal musculature of the larva into adult nerve innervating the DLM of the adult is well established for the tobacco hornworm, *Manduca sexta* [14].

The absence of dorsoventral muscles (DVM) in the sham-operated (mesothoracic leg disc extirpated) controls needs some explanation. In this group of control flies there is an intact larval mesothoracic nerve and therefore the DLM differentiate. However, the DVM fail to form. Similar results have also been reported earlier for *Drosophila melanogaster* [27] and *Sarcophaga bullata* [28]. We can suggest two possible explanations. (a) Since mesothoracic leg discs also contribute to the adult epidermis of ventral mesothoracic segment [29] the DVM of disc extirpated animals would have degenerated secondarily due to lack of ventral attachment sites (sclerites). Studies with *Sarcophaga bullata* [30] and *Drosophila melanogaster* [31] support this possibility. (b) The source

of myoblasts for DVM and DLM may be different. Investigations with *Drosophila* wing mutants support this view. In the mutant *wingless* development of DVM is affected while DLM are normally formed. In *erect wing* mutants, on the other hand, DLM are completely absent while DVM are normal [31].

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